In the Specification:

Please AMEND the specification as shown in the paragraphs below that begin on the page and line shown:

Page 11, line 27:

FIGs. [9A-9F] 9A-9D show that ovarian hormones alter *Hunk* mRNA expression *in vivo* in mammary glands and uteri of mice. Northern blots depict total RNA expression of tissues (mammary glands, FIG. 9A; or uteri, FIG. 9B), harvested from either intact females (sham) or oophorectomized females that received daily subcutaneous injections of either PBS carrier alone (OVX), 17b-estradiol (OVX+E$_2$), progesterone (OVX+P), or both 17b-estradiol and progesterone (OVX+E$_2$+P). Each sample represents a pool of samples hybridized overnight with ^{32}P-labeled antisense RNA probes specific for *Hunk* and *β-actin*. Signal intensities were quantified by phosphorimager analysis and *Hunk* expression was normalized to *β-actin* expression levels. *Hunk* expression relative to expression in oophorectomized (OVX) controls is shown below each lane. FIG. 9C depicts quantification of *Hunk* expression in mammary glands and uteri from intact FVB female mice after injection with PBS (control; light shaded boxes) or a combination of 5 mg progesterone in 5% gum arabic; and 20 μg of 17b-estradiol in PBS (+E$_2$+P; dark shaded boxes). RNase protection analysis was performed on either breast or uterus total RNA using ^{32}P-labeled antisense RNA probes specific for *Hunk* and *β-actin*. *Hunk* expression was quantified by phosphorimager analysis and normalized to *β-actin*. Values are shown relative to control animals. Each bar represents the average of 4 animals ±s.e.m. for each group. FIG. 9D depicts *in situ* hybridization analysis of *Hunk* expression in mammary gland sections from oophorectomized mice treated with hormones as described in FIG. 9A. Dark-field exposure times were identical in all cases. al, alveoli; d, duct; st, adipose stroma.

Page 15, line 9:

After kinases were clustered on the basis of similarities in their temporal expression profiles during mammary development, multiple distinct patterns of expression were observed. Analysis of these patterns revealed an ordered set of expression profiles in which successive waves of kinase expression occur during development. This resulted in the identification of a novel serine/threonine kinase of the present invention, the Hormonally Up-Regulated, Neu-

Tumor-[Asssociated] <u>Associated</u> <u>K</u>inase (HUNK). Originally referred to as Bstk1 (before being renamed Hunk), the kinase was first identified as a 207-bp RT-PCR product isolated from a mammary epithelial cell line derived from an adenocarcinoma arising in an MMTV-*neu* transgenic mouse (Chodosh *et al., Cancer Res.* 59:S1765–S1771 (1999)).

Page 16, line 13:

The 3'-UTR of *Hunk* is 2.8 kb in length, but lacks a canonical AATAAA polyadenylation signal (SEQID NO:4), containing instead the relatively uncommon signal, AATACA (SEQID NO:5), 18 nucleotides upstream from the poly(A)$^+$ tract (Bishop *et al., Proc. Natl. Acad. Sci. USA*, 83:4859–4863 (1986); Herve *et al., Brain Res. Mol. Brain Res.*, 32:125–134 (1995); Myohanen *et al., DNA Cell Biol.* 10:467–474 (1991); Myohanen *et al., DNA Seq.*, 4:343–346 (1994); Parthasarathy *et al., Gene,* 191:81–87 (1997); Tokishita *et al., [Gen,e] <u>Gene</u>* 189:73–78 (1997)).

Page 19, line 18:

The gene encoding Hunk kinase may be isolated as described herein, or by other methods known to those skilled in the art in light of the present disclosure. Alternatively, since, according to the present invention, the gene encoding Hunk has been identified, isolated and characterized, any other *Hunk* gene which encodes the unique protein kinase [deseibed] <u>described</u> herein may be isolated using recombinant DNA technology, wherein probes derived from *Hunk* are generated which comprise conserved nucleotide sequences in kinase gene. These probes may be used to identify additional protein kinase genes in genomic DNA libraries obtained from other host strain using the polymerase chain reaction (PCR) or other recombinant DNA methodologies.

Page 37, line 9:

Animals and Tissues. FVB mice were housed under barrier conditions with a 12-h light/dark cycle. The mammary glands from between 10 and 40 age-matched mice were pooled for each developmental point. Mice for pregnancy points were mated at 4–5 weeks of age. Mammary gland harvest consisted in all cases of the No. 3, 4, and 5 mammary glands. The lymph node embedded in the No. 4 mammary gland was removed prior to harvest. Tissues used for RNA preparation were snap frozen on dry ice. Tissues used for in situ hybridization analysis were embedded [inO.C.T.] <u>in O.C.T.</u> embedding medium (10.24% polyvinyl alcohol; 4.26%

polyethylene glycol) and frozen in a dry ice/isopentane bath. Developmental expression patterns for 13 kinases were confirmed using independent pools of RNA. Analysis of the developmental expression pattern for an additional kinase using these independent pooled samples revealed a similar pregnancy-up-regulated expression pattern that differed with respect to the day of pregnancy at which maximal up-regulation occurred.

Page 44, line 15:

GST–Hunk recombinant fusion proteins containing amino-[teminal] terminal (amino acids 32–213) or carboxyl-terminal (amino acids 556–714) regions of Hunk were expressed in BL21 bacterial cells and purified using glutathione– Sepharose beads according to the manufacterer's instructions (Pharmacia). Following removal of the GST (glutathione-S-transferase) portion by cleavage with Prescission Protease (Pharmacia, Piscataway, NJ), the liberated carboxyl-terminal Hunk polypeptide was further purified by isolation on a 15% SDS–PAGE gel.

Page 44, line 17:

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Page 51, line 5:

Example 3: Developmental Role of Hunk Kinase in Pregnancy-[Induced] Induced Changes in the [Mmammary] Mammary Gland

Animal and tissue preparation

FVB mice were housed under barrier conditions with a 12-hour light/dark cycle. Mammary glands from pregnant females were harvested at specified time points after timed matings. Day 0.5 was defined as noon of the day on which a vaginal plug was observed. Gestational stage was confirmed by analysis of embryos. Transgenic mothers were housed with

wild type mothers immediately after parturition to ensure pup survival and equivalent suckling stimuli. Both transgenic and wild type females were observed to nurse pups.

Page 52, line 4:

A full-length cDNA clone, G3, encoding *Hunk*, was digested with [~~mal~~] _Sma_I and _Spe_I to liberate a 3.2 kb fragment containing the complete coding sequence for *Hunk* (GenBank Accession number AF167987). This fragment was cloned downstream of the mouse mammary tumor virus long terminal repeat (MMTV LTR) into the multiple cloning site of pBS-MMTV-pA (Gunther, unpublished), which consists of the MMTV LTR upstream of the *H-ras* leader sequence (Huang *et al., Cell,* 27:245-255 (1981)) and SV40 splicing and polyadenylation signals. Linearized plasmid DNA was injected into fertilized oocytes harvested from superovulated FVB mice.

Page 52, line 26:

Four founder mice were identified harboring the MMTV-*Hunk* [~~transgenein~~] transgene in tail-derived DNA that passed the transgene to offspring in a Mendelian fashion. These were screened for transgene expression by Northern hybridization and RNase protection analysis. One founder line, MHK3, was identified that expressed the MMTV-*Hunk* transgene at high levels. Of note, a subset of transgene-positive MHK3 animals was found not to express the MMTV-*Hunk* transgene. All MHK3 non-expressing animals were analyzed by Southern hybridization analysis to confirm transgene presence and the expected MHK3-specific integration site.

Page 68, line 14:

Consistent with results obtained in lactating MHK3 animals, steady-state levels of lactoferrin mRNA were significantly higher in the mammary glands of nulliparous MHK3 expressing transgenic animals compared with either non-expressing MHK3 transgenic animals or age-matched nulliparous wild type animals, after normalization to *β-actin* (FIG. 14). This effect was surprising. Therefore to determine whether the effects of Hunk overexpression on lactoferrin expression may be more specific than the generally inhibited mammary epithelial differentiation that results from Hunk overexpression during pregnancy and lactation, gene expression patterns were compared in wild type and MHK3 nulliparous transgenic glands using oligonucleotide-based cDNA microarrays. These microarray studies revealed that, of the

approximately 5500 genes analyzed, the gene for lactoferrin is one of only 16 genes whose expression changes by more than 2.5-fold in transgenic animals, when compared with wild type glands. As noted above, the mammary glands of nulliparous MHK3 animals are morphologically indistinguishable from those of wild type littermates. Thus, the data indicate that the effects of Hunk [overexpressionon] overexpression on lactoferrin gene regulation are relatively specific, and are unlikely to be secondary to marked abnormalities in mammary gland morphology or to global changes in gene expression.

Page 68, line 18:

In contrast to lactoferrin, mRNA expression levels of the epithelial differentiation markers, β-casein, κ-casein, α-lactalbumin (Lalba – Mouse Genome Informatics, [location]), WDNM1 (Expi – Mouse Genome Informatics), and WAP, in adolescent nulliparous females, were not significantly affected by *Hunk* overexpression (FIG. 14, and data not shown). Consistent with this finding, the rate of ductal elongation and extent of epithelial side-branching in mammary glands from 5- to 6-week-old nulliparous transgenic mice was comparable with that observed in wild type mice, as analyzed by whole-mount and histological analysis (data not shown). These observations indicated that Hunk does not cause precocious differentiation of the mammary gland during puberty, but may specifically activate pathways resulting in *lactoferrin* upregulation. Similarly, the observation that lactoferrin expression is up-regulated in the mammary glands of lactating MHK3 animals, despite the global inhibitory effect of Hunk overexpression on mammary epithelial differentiation during late pregnancy and lactation, confirmed the conclusion that the effects of Hunk on lactoferrin expression are distinct from those on mammary epithelial differentiation.

Page 69, line 29:

RNA was isolated from 6 benign breast tissue samples and from 46 primary breast tumors obtained after surgery. An RNase protection analysis was performed using 10 μg of total RNA hybridized with a ^{32}P-labeled antisense riboprobe specific for *HUNK, cytokeratin 18 (CK18)* or for *β-actin. HUNK* and *β-actin* expression levels were quantified by phosphorimager analysis, and *HUNK* expression levels were normalized to either CK18 or [*β-actinfor*] *β-actin for* each sample. *HUNK* expression levels in breast tumors were compared with benign tissue. Normalized *HUNK* expression levels in the benign tissues was set equal to 1.0. This analysis

demonstrated that among all breast tumors, *HUNK* is expressed at a level that is 2.2-fold lower than in benign ovarian tissue. Moreover, when analyzed by subsets, 76% of all breast tumors were found to exhibit *HUNK* expression levels that were 5.0-fold lower than the average *HUNK* expression levels observed in benign tissue. Further analysis of *HUNK* expression as a function of breast tumor grade revealed that *HUNK* expression correlates negatively with tumor grade with poorly-differentiated (p=0.036) and moderately-differentiated (p=0.0029) tumors exhibiting lower levels of *HUNK* expression than benign tissues. Finally, expression of *HUNK* was also found to be decreased in both ductal carcinomas and lobular carcinomas.

Page 70, line 13:

In a similar manner, RNA was isolated from 16 benign ovarian tissue samples and from 22 primary ovarian tumors obtained after surgery. An RNase protection analysis was performed using 10 μg of total RNA hybridized with a ^{32}P-labeled antisense riboprobe specific for *HUNK* or for *β-actin*. *HUNK* and *β-actin* expression levels were quantified by phosphorimager analysis, and *HUNK* expression levels were normalized to [*β-actinfor*] *β-actin* for each sample. *HUNK* expression levels in ovarian tumors were compared with benign tissue. Normalized *HUNK* expression levels in the benign tissues was set equal to 1.0. This analysis demonstrated that *HUNK* is expressed in ovarian tumors at a level that is 10.3-fold higher than in benign ovarian tissue (p=0.0000034). Further analysis of *HUNK* expression as a function of ovarian tumor grade revealed that *HUNK* expression correlates positively with tumor grade with poorly-differentiated tumors and moderately-differentiated tumors exhibiting higher levels of *HUNK* expression than well-differentiated tumors.